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# SCIENTIFIC CRITERIA DOCUMENT FOR THE DEVELOPMENT OF INTERIM PROVINCIAL WATER QUALITY OBJECTIVES FOR 2,4-DINITROTOLUENE AND 2,6-DINITROTOLUENE

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# 2,4-DINITROTOLUENE

# and

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## PREFACE

The Ontario Ministry of Environment and Energy (OMOEE) develops Provincial Water Quality Objectives (PWQOs) for substances of environmental concern due to their potential persistence, bioaccumulation, toxicity and presence in Ontario surface waters. Ministry staff, directly responsible for surface water quality, also may request an evaluation.

In 1988, a wastewater pond system containing dinitrotoluene (DNT) overflowed into the St. Clair River from an industrial plant located at Courtright, Ontario. Water quality objectives were rapidly developed for 2,4-DNT and 2,6-DNT to assist ministry staff responding to the uncontrolled discharge. The objectives were subsequently used in a Certificate of Approval to limit further pond discharges from the site. By necessity, the original literature search and data assessment were not as thorough as possible. The purpose of this document was to complete the process, and fully develop PWQOs for the two DNT isomers.

PWQOs are numeric or narrative criteria intended to protect all stages of aquatic life for indefinite exposures. They also are intended to protect recreational uses of water (including swimming) based on microbiological and aesthetic considerations. They do not account for analytical detection limits, chemical treatability, socio-economic factors, natural background levels, or transport of contaminants between air, water and soil. These factors are considered in the policies and procedures for implementing PWQOs, contained in *Water Management* (OMOEE, 1994), which governs all aspects of Ontario's water management.

PWQOs represent a desirable water quality for the designated uses of surface waters in Ontario. They are used to help ensure that areas with satisfactory water quality are maintained, and areas with degraded water quality are improved. They may be used as the basis for effluent limits as specified in Certificates of Approval. Where better water quality is required to protect other beneficial water uses at a certain location, site-specific criteria including public health considerations, are taken into account.

The process for deriving PWQOs is documented in *Ontario's Water Quality Objective Development Process* (OMOE, 1992a). The scientific literature is reviewed for information on aquatic toxicity, bioaccumulation, mutagenicity and aesthetics (taste and odour of water and tainting of fish tissues). Where there is an adequate amount of reliable data, a PWQO is calculated by dividing the lowest effect concentration by a safety factor. If fewer data are available, an Interim PWQO is developed using a larger safety factor referred to as an *uncertainty factor*. The size of the uncertainty factor is determined by the quality and quantity of toxicity data available and by the potential of the chemical to bioaccumulate.

PWQOs do not consider analytical capability to detect the element or chemical of interest at the Objective concentration. As a result, PWQO for some parameters are currently below routine detection limits or may not be detectable under unusual sample matrix conditions. PWQO below detection create practical difficulties for water managers attempting to assess water quality against PWQO. There are several solutions to these difficulties including non-routine methods

such as large-volume water samples; or the analysis of an alternate environmental medium such as sediment or biological tissue to indirectly monitor water quality. Receiving-water concentrations may be estimated from known loadings (eg. from release inventories, effluent or groundwater concentrations). Bioassays may be conducted on effluent or receiving-water samples to infer water quality based on biological uptake, or biological surveys can be conducted on receiving waters. Nevertheless, where PWQO are below detection there may still be practical difficulties which will require site-specific judgement. Alternatively, it may be necessary to modify or develop analytical methods to measure lower concentrations in water. Decisions to develop analytical methods will be made by the Ministry in consultation with private and public sector laboratory stakeholders.

## ACKNOWLEDGEMENTS

The following OMOEE staff are acknowledged for their contributions to this report: Michael Salamone searched the literature and wrote the mutagenicity assessment appended to the document. Russell Fosberg provided the historical data for the St. Clair River water quality surveys. Chris Hutt, Tim Erme and Ted Tuszynski commented on Ontario sources and effluent discharges.

Peer review comments on the draft report were invited from forty-seven stakeholders including seventeen OMOEE groups, twenty-one provincial and federal agencies, four U.S. agencies, three academics and two industrial sector groups. Review comments were received from: Glenn Rutherford, Roy Angelow and Peter Kauss (MOEE), Paul Shewchuk (Alberta Environmental Protection), Narender Nagpal (British Columbia Ministry of Environment, Lands and Parks) and Kevin Buhl (United States National Biological Service). Isabelle Guay (Gouvernement du Québec Ministère de l'Environnement et de la Faune) provided a list of Quebec's interim aquatic criteria for the nitrotoluenes.

## EXECUTIVE SUMMARY

The purpose of this document was to develop Interim PWQOs for 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT). The chemical compounds were selected because they had been identified as hazardous substances detected in Ontario effluents and surface waters. Literature information on their physical-chemical properties, aquatic toxicity, bioconcentration and aesthetics were available to derive the objectives.

DNT is synthesized as a technical-grade product that contains 2,4-DNT (77%), 2,6-DNT (19%) and four other DNT isomers (4%). The product is used to make explosives, urethane polymers, flexible and rigid foams, surface coatings and dyes and it is used in organic chemical syntheses. There are no identified point source discharges in Ontario, but trace amounts of DNT are occasionally detected in effluents and surface waters. Detectable traces probably result from incidental formation where small amounts of toluene and nitric acid inadvertently come into contact, and from small-volume commercial and industrial uses of the DNT product. Biodegradation of trinitrotoluene (TNT) produces DNT as a metabolic product, another possible source of trace levels in the environment.

DNT is nonvolatile and water soluble. Its aquatic environmental distribution and fate would be determined by dilution, dispersion and reaction processes. Photolysis and biodegradation are the primary breakdown processes that prevent DNT from accumulating in the aquatic environment. DNT is not bioaccumulative. Mutagenicity data on aquatic life were not available, but both compounds were weak to moderate mutagens in bacterial and mammalian assay systems.

Plants and protozoa were consistently the most sensitive groups to 2,4-DNT: toxicity was observed at water concentrations less than 5 mg/L for 11 of the 12 test species. Acute toxicity to fish and invertebrates occurred at concentrations ranging from 8 to 100 mg/L. Chronic toxicity generally occurred at concentrations ranging from 0.5 to 31 mg/L. The lowest effect concentration was 50 ug/L for growth inhibition of bluegill sunfish. **The recommended Interim PWQO for 2,4-DNT is 4 ug/L**. It was calculated by dividing the 50 ug/L toxicity value by a final uncertainty factor of 13.

Few data were available for 2,6-DNT, but acute and chronic effects on ten species including fish, invertebrates, algae, protozoa and bacteria occurred at concentrations ranging from 1 to 34 mg/L. The lowest effect concentration was 1 mg/L for growth inhibition of *Daphnia magna*. **The recommended Interim PWQO for 2,6-DNT is 6 ug/L**. It was calculated by dividing the 1 mg/L toxicity value by a final uncertainty factor of 179.

Analytical detection limits are significantly lower than the Interim PWQOs, so water concentrations can be quantified for comparison to the objectives. The aesthetic qualities of water and fish tissue should not be impaired by DNT at the PWQO level, but few data were available on aesthetics.

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## 1.0 INTRODUCTION

Dinitrotoluene or DNT ($C_6H_3CH_3(NO_2)_2$) is synthesized by nitrating toluene with sulphuric and nitric acid. The commercial product is technical-grade DNT (tDNT), a mixture comprised of six DNT isomers (see Table 1). DNTs are nitro-aromatic compounds, and are also known as dinitrotoluols or methyl-dinitrobenzenes. They are orange-yellow solids at room temperature. The tDNT product is used to make explosives (trinitrotoluene or TNT), urethane polymers, flexible and rigid foams, surface coatings, propellants and dyes and other commercial chemicals, notably 2,4-diaminotoluene (CCREM, 1987; Anon, 1987; Sittig, 1985).

Table 1. *Isomeric composition of technical-grade DNT.*[1]

| **DNT isomer** | 2,4-meta | 2,6-meta | 3,4-ortho | 2,3-ortho | 2,5-para | 3,5-meta |
|---|---|---|---|---|---|---|
| **% of tDNT** | 76.5 | 18.8 | 2.4 | 1.5 | 0.7 | 0.04 |

1 Anon (1987)

The purpose of this document was to develop PWQOs for 2,4- and 2,6-DNT, two *meta*-isomers that comprise about 95 % of tDNT. The substances were selected because they are hazardous and they have been detected in Ontario effluents and surface waters. PWQOs were not developed for the other four DNTs because they have not been detected in Ontario waters. Although the other isomers comprise less than 5% of tDNT, the literature suggests that the *ortho*- and *para*-isomers are about ten times more toxic than the *meta*-isomers. The comparative toxicity data on the various DNT isomers are discussed in section 2.4.

Mutagenicity data on aquatic species were unavailable, but both DNT isomers were characterized as weak to moderate mutagens in mammalian and bacterial assay systems. A review of this DNT mutagenicity literature is provided in the appendices. The potential effects of 2,4-DNT and 2,6-DNT on human health and the environment have been reviewed by the United States Environmental Protection Agency (USEPA, 1986). Mixtures of the two DNT isomers and 2,4-DNT alone are listed by the USEPA as *probable human carcinogens* with a cancer rating of *B2* (IRIS, 1992).

### 1.1 Analytical methods

Before 1988, the routine isomer-specific analytical detection limit in water was 1.0 ug/L for both compounds. Since then, the detection limits have been as low as 0.05 ug/L for 2,4-DNT and 0.01 ug/L for 2,6-DNT, using chromatography and an electron capture detector. If water samples contain other similar compounds that interfere with the analysis, as frequently occurs with complex effluents, detection limits may be up to ten times higher. In Ontario's industrial effluent monitoring regulations, the regulatory detection limits were 0.8 ug/L for 2,4-DNT and 0.7 ug/L for 2,6-DNT (OMOE, 1992b). The 2,4-DNT isomer is more difficult to separate and measure

due to its chromatographic behaviour. The concentration of 2,4- and 2,6-DNT in water at the PWQO is thus readily measurable by most routine sampling and laboratory test methods.

### 1.2 Sources and ambient levels in Ontario waters

DNT may enter the aquatic environment directly in a wastewater discharge or indirectly from a commercial or industrial discharge to a municipal sewer. Other potential sources include spills and leaks associated with production, use, storage and disposal activities. Historically, 2,4-DNT and 2,6-DNT were detected in the effluents of chemical industries on the St. Clair River. In 1979 and 1980, 2,4-DNT was measured in three of six effluent samples at 10 to 100 ug/L, and 2,6-DNT was detected in three of thirteen samples (CCREM, 1987; Munro *et al.*, 1985). Effluent monitoring in 1989 to 1991 detected 2,6-DNT in both the intake water and the effluents at one industrial site (OMOE, 1992b). The source of the DNT was unknown. It could have been formed incidently at an industrial site if small amounts of toluene and nitric acid inadvertently came into contact during chemical tank washing. Small-volume commercial and industrial uses of DNT are also possible sources. DNT also is formed by biological degradation of TNT (see section 1.2).

In 1988, a wastewater pond system containing DNT overflowed into the St. Clair River from an industrial plant located at Courtright, Ontario. The plant produced ammonium phosphate fertilizer by reacting sulphuric acid with phosphate rock to obtain phosphoric acid. The phosphoric acid was then reacted with ammonia to produce the ammonium phosphate. The process wastewater was stored on-site in ponds and the DNT originated as an impurity in the *spent* sulphuric acid. After industrial production stopped in 1986, the ponds continued to fill with water from precipitation, leading to the 1988 overflow into the St. Clair River. Further discharges from the ponds were limited to 4 ug/L 2,4-DNT and 3 ug/L 2,6-DNT, as specified in a Certificate of Approval. The DNT in the ponds has since degraded by natural processes, and it is no longer detectable (detection limit 0.5 ug/L).

In Ontario surface waters, 1979 and 1987 surveys found traces of 2,4-DNT and 2,6-DNT in the St. Clair River (CCREM, 1987; OMOE, 1987). In a 1988 survey, following the industrial pond overflow described above, 2,4-DNT was not detected in thirty-six samples collected at several sites along the river (OMOE, 1988).

### 1.2 Environmental properties and fate

Literature values of the physical-chemical properties are listed in Table 2 (2,4-DNT) and Table 3 (2,6-DNT). As expected, the two isomers have similar properties. Based on the Henry's Law Constants, water to air transfer by evaporation would be an insignificant environmental pathway. The solubility parameter *Kow* refers to the octanol-water partition coefficient, usually expressed on a logarithmic scale (log Kow). Compounds with log Kow values above four are a concern due to their potential to bioaccumulate and persist in the aquatic environment (OMOE, 1992a). All the log Kow literature values were within the range 1.7 - 2.3 for both isomers, so DNT would

not be expected to accumulate in the sediment or in the aquatic food chain. Therefore, DNT released to surface water would be expected to remain in the water column.

A literature review of DNT fate processes (USEPA, 1986) concluded that evaporation, particle-binding and sedimentation were not significant. Biodegradation and photolysis were identified as the primary processes preventing accumulations of DNT in the aquatic environment. Photolysis half-life times of 43 hours in distilled water and 3 to 10 hours in natural waters were reported in the literature. Biodegradability was assessed in laboratory tests of biochemical oxygen demand (BOD). The tests had variable results depending on experimental conditions. Both DNT isomers, at concentrations from 10 to 200 mg/L, were rapidly degradable by microbial inoculum from the sludge of an industrial wastewater treatment system. Microbial metabolites included 4-methyl-3-nitroaniline from 2,4-DNT and 2-methyl-5-nitroaniline from 2,6-DNT (Davis *et al.*, 1981).

The two DNTs were found to be inhibitory or toxic to inocula from municipal sewage treatment plants. Tabak *et al.* (1981) found both DNTs were inhibitory at 5 and 10 mg/L. Davis *et al.* (1981) found inhibition by 2,4-DNT concentrations as low as 10 mg/L, and by 2,6-DNT at or above 50 mg/L. One implication is that a *slug* loading of DNT into a municipal sewer could pass through the treatment plant or upset the biological treatment process.

Niemi *et al.* (1987) compared the structures of organic compounds to the results of BOD tests. Mono-aromatic compounds with hydrophillic substituents, like DNT, were classified as degradable, with half-life times from 2 to 16 days.

Haïdour and Ramos (1996) reviewed the literature and noted that several microorganisms can use DNT as a food source. In a laboratory study, they identified chemical products resulting from the metabolism of TNT, 2,4-DNT and 2,6-DNT by a *Pseudomonas* bacterium species. The metabolism involved two reduction processes. One was the progressive removal of nitro groups from the aromatic ring such that 2,4-DNT was identified as a metabolite of TNT. In the other process, TNT and the DNTs were metabolized to monoamino-mononitrotoluene derivatives. The reduction of the DNTs probably occurred via hydroxylamino intermediates, since all possible azoxytoluenes (from the condensation of two aromatic rings) also were found. The metabolic products were recalcitrant and accumulated in the bacterium culture medium.

Bailey (1982) reported the following half-life times for 2,4-DNT in the water column of a laboratory microcosm: 2 days for 1 mg/L, 4 days for 10 mg/L, and 7.5 days for 56 mg/L. The author noted, for an unspecified concentration, that a theoretical half-life of 11 hours in natural waters was predicted by studies that measured individual degradation processes and integrated the results into an overall degradation rate. Zoeteman *et al.* (1980) reported a half-life time of 1.7 days for 0.3 ug/L of 2,4-DNT in the Rhine River.

In summary, the weight of evidence suggests the DNT isomers are not persistent in the aquatic environment, primarily due to breakdown by photolysis and biodegradation.

## 2.0 TOXICITY TO AQUATIC ORGANISMS

The literature toxicity data on DNT were primarily from a hazard assessment conducted by the United States Army about wastewaters from munitions plants. The toxicity data from this assessment were published in: Pearson *et al.* (1979), Hartley (1981), Liu *et al.* (1983a; 1983b), Bailey (1982) and Bailey and Spanggord (1983). Where the same test result was cited in more than one of these references, it was considered the same data. Where a test result differs slightly between papers both results were reported here, recognizing that it may be from the same test only with a difference in statistical analysis (note: this potential duplication of listed data does not affect the objective-setting exercise). Liu *et al.* (1983a) provided the most extensive aquatic toxicity data for 2,4-DNT. Therefore the discussion below was based on the 2,4-DNT data from this reference, unless otherwise indicated.

The toxicity data, discussed below, are fully reported in Table 4 (2,4-DNT) and Table 5 (2,6-DNT), and are plotted in Figure 1 (2,4-DNT) and Figure 2 (2,6-DNT). Toxicity data on the other DNT isomers are summarized in sections 2.3 and 2.4. The database includes the *toxicity threshold* values for the inhibition of cell multiplication of bacteria, algae and protozoa (Bringmann and Kühn, 1977; 1978; 1980; 1981 and 1982). A toxicity threshold is an estimate similar to a *no observed effect concentration* (NOEC) compared to the control treatment. The authors noted that the toxicity test concentrations differed by a factor of two, so doubling a threshold value would estimate a *lowest observed effect concentrations* (LOEC) compared to the control treatment. These threshold values were included in this document, but they were unacceptable for deriving objectives because PWQOs are based on toxic effect concentrations only.

### 2.1 Acute toxicity

#### 2.1.1 Vertebrates

In a comparative study of four fish species, the median lethal concentrations (LC50s) for 2,4-DNT ranged from 8 to 38 mg/L. The LC50s were not significantly different for static and flowthrough tests, but the 4d-LC50s were slightly lower than the 1d-LC50s. Bluegill sunfish (*Lepomis macrochirus*) was the most sensitive fish (4d-LC50 8 mg/L). The guppy (*Poecelia reticulata*) 4d-LC50 was 25 mg/L in another study (Adema *et al.*, 1983). Sensitivity differences were within a factor of two among all five fish species. Bluegill sunfish acute lethality tests were conducted under various conditions of water quality. The 4d-LC50 was three times lower at 24°C than at 17°C for waters of similar hardness and pH. Lethality was not modified by water hardness (40-240 mg/L as $CaCO_3$) or by pH (6.3-8.0).

The acute toxicity of photolyzed 2,4-DNT was investigated. A stock solution was irradiated with filtered ultra-violet light, and the amount of 2,4-DNT remaining in the stock solution was measured (detection limit 50 ug/L) to indicate the extent of photolysis. A fathead minnow (*Pimephales promelas*) static acute lethality test was conducted on an irradiated stock solution,

and the LC50 was expressed as a nominal concentration based on the original amount of 2,4-DNT. Other test conditions were unspecified. 2,4-DNT was photo-sensitive, and the breakdown products were less toxic than the parent compound (Table 6).

Table 6. *Lethality of photolyzed 2,4-DNT.*[1]

| Percent photolysis | 96h-LC50 (mg/L) | 95 % confidence limits (mg/L) |
|---|---|---|
| 0 | 31 | 29 to 34 |
| 50 | >35 | not calculated |
| 100 | 65 | 56 to 75 |

1 Liu et al., (1983a)

For 2,6-DNT, 4d-LC50s were 18.5 and 19.8 mg/L in fathead minnow acute lethality tests.

### 2.1.2 Invertebrates

2,4-DNT acute toxicity data were available for three invertebrate species. Ten toxicity values were available for the cladoceran *Daphnia magna*, tested under different conditions. The LC50s and EC50s (immobilization) ranged from 10 to 48 mg/L. Toxicity was unrelated to the type of test (static or flowthrough), exposure time (one or two days), water pH (7.5 to 8.4) or water hardness (20 to 200 mg/L). 2,6-DNT toxicity data were available for *D. magna*. The LC50s and EC50s ranged from 14 to 34 mg/L in four tests.

## 2.2 Chronic toxicity

### 2.2.1 Vertebrates

Incipient lethal levels of 2,4-DNT, LC50s estimated after mortality did not increase further with longer exposure, ranged from 6 to 26 mg/L in a comparative study of four fish species. Rainbow trout was the most sensitive (14d-LC50 6.3 mg/L) and fathead minnow the least sensitive (14d-LC50 26 mg/L) fish. Deneer *et al.* (1987) reported a 14d-LC50 of 12.6 mg/L for the guppy.

Hartley (1981) reported on two chronic tests with juvenile bluegill sunfish. Fish body weights were measured weekly over 56 days of exposure to 2,4-DNT, and fish were dissected and examined for tissue damage. The cumulative growth rate over time at each test concentration was analyzed using established regression models, and the growth rates were compared using statistical tests of significance. In the first test, five fish were exposed to concentrations of 8.0, 5.0, 2.0, and 0.5 mg/L and a control. Growth was completely inhibited at 5 and 8 mg/L, and was depressed at 2 mg/L and 0.5 mg/L.

In the second test, 25 fish were exposed to concentrations of 8.0, 5.0, 4.0, 2.0, 1.0. 0.5, 0.05 mg/L and a control. Growth depression was observed for fish exposed to the lowest concentration, 0.05 mg/L, and a concentration-related increase in effect was observed up to 5 mg/L. The study noted, *the actual threshold may be a little less than 0.05 mg/L.* No histological abnormalities were found in the digestive tract, pancreas, integument, heart, gonad, head kidney and spinal cord. Significant abnormalities were found in the liver, spleen, trunk kidney, lateral line and gill in fish exposed to concentrations ≥ 0.5 mg/L.

In chronic toxicity studies of fathead minnow, 2,4-DNT had no significant effect on egg survival. 7 mg/L of 2,4-DNT significantly reduced the number of viable fry emerging from eggs. A concentration of 2 to 4 mg/L inhibited fry growth (body length) after 30 days of exposure, and survival was significantly reduced at 7 mg/L after 90 and 150 days of exposure (unpublished data cited in Hartley, 1981). For 2,6-DNT, a guppy lethality test resulted in a 14d-LC50 of 18 mg/L (Deneer *et al.*, 1987).

### 2.2.2 Invertebrates

A chronic lethality test of *D. magna* resulted in an incipient LC50 of 4 mg/L for 2,4-DNT. Deneer *et al.* (1989) reported 0.6, 1.0 and 1.0 mg/L as 21d-LOECs for *D. magna* lethality, population growth and body length, respectively. For 2,6-DNT, a 21d-LC50 of 9.6 mg/L and 21d-LOECs of 10 mg/L (population growth) and 1.0 mg/L (body length) were reported for *D. magna* (Deneer *et al.*, 1989).

### 2.2.3 Aquatic Plants

Aquatic plants were sensitive to 2,4-DNT. 11d-LOECs were 0.1 and 1.0 mg/L for colony growth of duckweed, *Lemna perpusilla* exposed to 2,4-DNT (Schott and Worthley, 1974). The bluegreen alga, *Microcystis aeruginosa*, was the most sensitive of seven algal species tested. Cell multiplication was inhibited at 0.5 mg/L in a 4-day and 14-day exposure, and the toxicity threshold was 0.13 mg/L (Bringmann and Kühn, 1978). *M. aeruginosa* was the most sensitive of three algae species tested with 2,6-DNT; the toxicity threshold was 0.5 mg/L (Bringmann and Kühn, 1978).

### 2.2.4 Micro-organisms

Data on 2,4-DNT toxicity were available for three protozoa and one bacterium (Bringmann and Kühn, 1980; 1981; Deneer *et al.*, 1989). The protozoa were sensitive to the toxic effects of 2,4-DNT. 0.13 mg/L was the threshold for inhibition of cell multiplication in a test of *Chilomonas paramecium.* For 2,6-DNT, toxicity data were available for three species of protozoa and one bacterium (Bringmann and Kühn, 1980; 1981; Deneer *et al.*, 1989). None was a notably sensitive species.

## 2.3 Mechanisms of toxic action

The toxic mechanisms of DNT can be inferred by an analysis of quantitative structure-activity relationships (QSARs) for nitro-aromatic compounds. A QSAR is a predictive correlation of a chemical's toxicity with its structure and related physical-chemical properties. A QSAR can be used to infer a certain mode of toxic action. For example, a near direct proportionality between toxicity and Kow for a group of compounds implies that the target site has nonpolar (lipid-like) properties consistent with a narcotic mode of action. Bioconcentration is so rapid that organisms cannot metabolize and excrete the chemical, and the normal pharmacokinetic differences among species are usually insignificant to the toxicity. Compounds more toxic than predicted by a QSAR for narcosis probably act by binding to specific receptors in target tissues. Some toxicants bind directly to target receptors, others are converted to the toxicant by enzymatic transformation (*bioactivation*). Small differences in chemical structure, as for the six DNT isomers, would not significantly alter their potency as narcotics, but could greatly alter their biotransformation and potency if they were specific-acting toxicants.

Pearson *et al.* (1979) lists acute toxicity values of twenty-nine nitro-aromatic compounds for fathead minnow lethality and *D. magna* immobilization. As noted in Bailey and Spanggord (1983), toxicity increased with an increasing number of nitro group substitutions on the aromatic ring. Among the DNT isomers, compounds with the nitro groups positioned *ortho* or *para* to each other were generally ten times more toxic than the *meta* isomers (Table 7).

Table 7. *Comparative acute toxicity of DNT isomers.*[1]

| Test end-point (mg/L) | 2,4-meta | 2,6-meta | 3,5-meta | 2,3-ortho | 2,5-para | 3,4-ortho |
|---|---|---|---|---|---|---|
| *D. magna* 2d-EC50 | 35 | 22 | 45 | 5 | 3 | 3 |
| minnow 4d-LC50 | 33 | 20 | 22 | 2 | 1 | 2 |

1 Pearson *et al.* (1979)

The relationship between chemical structure and toxicity was attributed to the rate of enzymatic transformation (nitro group reduction) of the DNT into more toxic intermediates (nitroxy-free radicals, hydroxlamine and/or nitroso compounds) that act by binding to biological macromolecules. The authors postulated that the *ortho* and *para* compounds had higher rates of bioactivation, and thus had higher acute lethality.

Deneer *et al.* (1987) compared the bioconcentration factors (BCFs) and lethality of twenty-three nitro-aromatic compounds using 14-day tests of the guppy. The uptake and effects of the mononitro compounds were adequately estimated from a correlation with Kow alone (suggesting narcosis). The dinitro compounds had lower BCFs and higher toxicities than predicted by Kow. The toxicity correlation was improved by including a second, kinetic parameter to account for the reduction potential of the nitro group. This suggests toxic intermediates were formed by enzymatic reduction of the parent dinitro compounds. 2,4-DNT and 2,6-DNT were exceptions.

They were more toxic than predicted by Kow alone, but significantly less toxic than predicted by the two-parameter correlation. The measured BCFs were slightly higher the BCFs estimated by Kow alone. The authors postulated that nitro group reduction was contributing to the fish lethality of the two DNT isomers, but the reduction rates were relatively slow compared to the rates of the other dinitro compounds.

Deneer *et al.* (1989) compared the acute and chronic toxicity of twenty-two nitro-aromatic compounds using three species. As before, narcosis was identified as the mode of action of the mononitro compounds. Bioactivation was postulated as the reason for the higher toxicity of the dinitro compounds. *D. magna* responses were used extensively in the study. The DNT isomer results for the daphnid tests are summarized in Table 8. *Ortho* DNT was more toxic than *meta* DNT for acute immobilization, as was found for fathead minnow acute lethality. For the chronic responses there was no consistent toxicity relationship among isomers, except 2,6-DNT was generally the least toxic. *D. magna* chronic responses were sensitive. The acute-chronic ratio (ACR) for immobilization was 56 (34/0.6) for 2,4-DNT, indicating that the sublethal response increased significantly as the exposure period increased.

Table 8. *Acute and chronic toxicity of DNT isomers to D. magna.*[1]

| **Toxicity end-point (mg/L)** | **2,4-meta** | **2,6-meta** | **2,3-ortho** | **3,4-ortho** |
|---|---|---|---|---|
| 48h-EC50 (immobilization) | 34 | 34 | 6 | 6 |
| 21d-EC50 (immobilization) | 0.6 | 10 | 2 | 1 |
| 21d-LOEC (pop. growth rate) | 1 | 10 | 3 | 0.3 |
| 21d-LOEC (body length) | 1 | 1 | 1 | 0.3 |

1 Deneer *et al.* (1989)

In the *L. macrochirus* tests of 2,4-DNT (Hartley, 1981; Liu *et al.*, 1983a), the ACR (14d-LC50/56d-LOEC) was 184 (9.2/0.05), where the chronic response was fish growth. For *P. promelas* lethality, the ACR (14d-LC50/150d-LOEC) was 4 (Hartley, 1981). Based on Liu *et al.* (1983a), the ACR (4d-LC50/14d-LC50) ranged from 1.4 to 2.2 for four fish species, and the highest value was 5.8 for *D. magna*. The low ACRs suggests that narcosis probably dominated the lethality response, since they did not increase much with longer exposures.

Overall, the evidence suggests that the toxicity of 2,4-DNT and 2,6-DNT has a narcotic component, especially in acute exposures, but the primary mechanism of chronic sublethal toxicity involves bioactivation to form reactive intermediates. *Meta* DNT probably had slower bioactivation (and/or faster detoxification) compared to the *ortho* and *para* isomers. Hence they were less toxic in the acute tests, but significant sublethal toxicity was eventually expressed in the chronic tests. The contribution of bio-transformation products to the toxicity of DNT would be expected to increase over time as the products and the tissue damage accumulate to a level that is manifested as effects on the whole organism.

## 3.0 MUTAGENICITY

Mutagenicity data on aquatic organisms were not available for either DNT. A qualitative assessment of bacterial and mammalian data concluded that both compounds were weak to moderate mutagens in various assay systems. Appendix A summarizes the mutagenicity literature, and Appendix B is a list of the literature references.

## 4.0 BIOCONCENTRATION

The uptake and excretion of 2,4-DNT was rapid in fish. In *P. reticulata*, tissue levels reached a steady-state within 6 hours (Deneer *et al.*, 1987). Tissue levels in *L. macrochirus* were nondetectable after 3 to 4 days of depuration in clean water (Hartley, 1981; Liu, *et al.*, 1983a; 1983b). Table 9 lists the data: the highest concentrations of DNT were found in brain tissue with intermediate levels in the gills, kidney, gastrointestinal tract and liver (Hartley, 1981). The lowest levels were in muscle tissue. The BCF values were less than one hundred for whole fish and invertebrates. The high BCFs for *Selenastrum capricornutum* (Liu *et al.*, 1983a; 1983b) may be due to chemical sorption to the outer cell wall rather than uptake into the cells by partitioning. For 2,6-DNT, a 4d-BCF of 22 was reported for *P. reticulata*.

## 5.0 TASTE AND ODOUR

No data were available about the taste and odour of 2,4-DNT in water. The water odour threshold of 2,6-DNT was 0.1 mg/L (Verschueren, 1983). 0.1 mg/L divided by the standard application factor of 2 gave 50 ug/L as an objective to protect water from odour caused by 2,6-DNT. This value is higher than the value to protect aquatic life derived below.

## 6.0 DERIVATION OF PROVINCIAL WATER QUALITY OBJECTIVES

OMOE (1992a) specifies the data requirements and PWQO-setting procedures for aquatic toxicity, bioaccumulation, taste and odour and mutagenicity considerations. Insufficient data were available for the DNT isomers, so Interim PWQO values were derived. Section 9.0 (Research Needs) identifies the aquatic toxicity data that are needed to set more scientifically-defensible objectives.

The Federal-Provincial Working Group on recreational water quality has not recommended limits for chemicals in recreational water for human exposure because of the lack of sufficient scientific information (Health and Welfare Canada, 1992). Therefore, a recreational use water quality objective for the protection of human health was not recommended here.

A PWQO should account for the possibility that seasonal and other variations in receiving water quality could affect toxicity. 2,4-DNT was found to be more toxic to duckweed at pH 6.3 than at

pH 8.5 (Schott and Worthley, 1974), and bluegill sunfish acute lethality was higher at 25 °C than at 17 °C (Liu *et al.*, 1983a). However this scant information was insufficient to modify a PWQO to account for water quality. Therefore the objective was set as a single value, independent of water temperature, pH and hardness. The photo-products of 2,4-DNT were less acutely toxic than the parent compound, so there was no basis for a more stringent PWQO to account for toxicity modified by photolysis.

Separate from the bioactivation discussed in section 2.3, there is the possibility that DNT could be biotransformed and eliminated as compounds more toxic than the parent DNT. Liu *et al.* (1983) found that static solutions of 2,4-DNT were more acutely toxic than flow-through solutions tested on six species. The authors noted that toxic biotransformation products might be accumulating in the water, but this was deemed unlikely because: (a) the difference in species sensitivity was much greater than the difference in the toxicity of the static and flow-through solutions; (b) two biotransformation products that were detected (amino-nitrotoluenes) in water had acute LC50s to fathead minnows and *D. magna* in the same range as the LC50s for 2,4-DNT and 2,6-DNT and; (c) in an unpublished environmental fate study, 2,4-DNT was rapidly mineralized by microorganisms. Davis *et al.* (1981) identified two isomers of methyl-nitroaniline as DNT biotransformation products, and speculated that the products might be hazardous, but this could not be substantiated. Thus there was no basis for modifying the PWQO value to account for this factor.

Since the six DNT isomers occur together in the technical-grade product, PWQO development should consider the possibility that objectives only for 2,4-DNT and 2,6-DNT might not be sufficiently protective. Acute toxicity tests found that the *ortho* and *para* DNT isomers were about ten times more toxic than 2,4-DNT and 2,6-DNT. Four approaches are possible to set water quality objectives:

(i) develop an objective for each of the six DNT isomers. Since the toxicity data were minimal for the other four DNTs, this approach would not be defensible. Also, the other isomers have not been detected in Ontario effluents or surface waters.

(ii) use an additive toxicity model to develop a sum-parameter objective for *tDNT*, the actual chemical formulation. Without extensive additional data, one would have to assume that the six DNT isomers have similar pharmacokinetic characteristics and modes of action in all species. Given the significant diversity in bioactivation enzyme systems and other biological characteristics among aquatic species, their responses to the six DNT would be expected to vary significantly. The limited evidence already suggests that the assumptions for additive toxicity do not apply in this case, so an objective for tDNT would not be defensible.

(iii) develop the same objective for 2,4-DNT and 2,6-DNT. The toxicity data for *Daphnia* and the QSAR analysis suggest the two isomers are about equally toxic. Unfortunately, toxicity data are lacking on the chronic sublethal effects of 2,6-DNT on fish, so it was not possible to reach a firm conclusion in this regard.

(iv) develop a separate objective for 2,4-DNT and 2,6-DNT. The toxicity database on 2,4-DNT represents a diversity of aquatic life and includes sensitive chronic sublethal responses. The toxicity database on 2,6-DNT was scant but adequate for setting an interim objective, using a larger uncertainty factor to compensate for the lack of information. Therefore separate objectives for 2,4-DNT and 2,6-DNT are feasible and appropriate.

### 6.1 Calculation of the final uncertainty factor

The selection of a baseline uncertainty factor depends on the bioconcentration potential of the compound. The range of log Kow literature values (1.7 to 2.3) for the DNT isomers was well below the log Kow *cut-off* value of four. The fish tissue residue data show that DNT is not significantly bioconcentrated. Therefore, a baseline uncertainty factor of 1000 was selected for both compounds. This factor was reduced to a final uncertainty factor according to the strength of the aquatic toxicity database (the data quality, the diversity of species tested and the availability of data on chronic and sublethal responses). The calculation of the final uncertainty factor is shown in Table 10 (2,4-DNT) and Table 11 (2,6-DNT).

The 2,4-DNT aquatic toxicity database was strong, as reflected by the final uncertainty factor of 13, which is the minimum value that can be derived. Several lethality tests were acceptable as chronic data because the tests were continued until there was no further increase in mortality with longer exposures. Also the ACR values for lethality were low, indicating little increase in mortality over time. Twenty species are represented in the database: five fish, three invertebrate, eight plant and four micro-organism species. The acute toxicity values spanned about one order of magnitude. The chronic and sublethal data spanned about three orders of magnitude. Plants and protozoa were generally the most sensitive taxonomic groups; the toxicity values were less than 5 mg/L for eleven of twelve test species. The most sensitive animal species were *D. magna* and *L. macrochirus* - chronic sublethal LOECs were $\leq$ 1.0 mg/L.

The 2,6-DNT aquatic toxicity database was relatively weak, so a larger final uncertainty factor (179) was derived to provide assurance that an interim PWQO would be protective. Toxicity data were available for ten species: two fish, one invertebrate, three plant and four microorganism species.

### 6.2 Calculation of the Interim PWQO

*L. macrochirus* growth inhibition at 50 ug/L (Hartley, 1981) is the critical toxicity value for setting an Interim PWQO for 2,4-DNT. 50 ug/L divided by the final uncertainty factor of 13 gives 4 ug/L as the objective to protect aquatic life from the toxic effects of 2,4-DNT.

Table 5 lists 0.5 mg/L as a *toxicity threshold* value (inhibition of cell multiplication) for *M. aeruginosa* (Bringmann and Kühn, 1978) exposed to 2,6-DNT. As noted previously, this is an

estimate similar to a NOEC, and it is unacceptable for deriving an objective. Therefore, *D. magna* growth (body length) inhibition at 1 mg/L (Deneer *et al.*, 1989) is the critical toxicity value for setting an Interim PWQO for 2,6-DNT. 1 mg/L divided by the final uncertainty factor of 179 gives 6 ug/L as the objective to protect aquatic life from the toxic effects of 2,6-DNT.

The final step is to compare the objectives to literature NOEC values to confirm that the objectives are protective of aquatic life. Adema *et al.* (1983) used NOECs to summarize the results of 2,4-DNT toxicity tests with *Scenedesmus pannonicus*, *D. magna*, *P. reticulata* and *Jordanella floridae*. Table 12 lists the NOECs from this study and other published NOECs for duckweed and *M. aeruginosa*, plant species sensitive to 2,4-DNT. The objective recommended for 2,4-DNT (4 ug/L) is lower than the NOEC values. A similar comparison could not be conducted for the 2,6-DNT objective.

Table 12. *NOEC values for 2,4-DNT.*[1]

| Test organism | Duration (days) | Response | NOEC (mg/L) |
|---|---|---|---|
| *S. pannonicus* | 4 and 14 | cell multiplication | 0.32 |
| *D. magna* | 2 | mobility | 10 |
| | 14 and 21 | mortality, reproduction, growth | 0.32, 1.0[2] |
| *P. reticulata* | 4 | mortality, swimming behaviour, colour (28d-old fish) | 3.2 |
| | 28 | mortality, growth swimming behaviour, colour | 1.8, 3.2[2] |
| *J. floridae* | 42 | egg-larval development, mortality, growth swimming behaviour, colour | 1.0 |
| *L. perpusilla* | 11 | colony growth at pH 8.5 | 0.1[3] |
| | | colony growth at pH 6.3 | 0.01[3] |
| *M. aeruginosa* | 4 and 14 | cell multiplication | 0.1[4] |

1 Adema *et al.* (1983); 2 separate tests; 3 Schott and Worthley (1974); 4 Liu et al. (1984).

## 7.0 RESEARCH NEEDS

Insufficient data on aquatic toxicity were available to develop PWQOs. Further information is needed on chronic sublethal (growth and reproduction) effects. For 2,4-DNT, toxicity test results are required for another fish (early life stage) and another invertebrate species. For 2,6-DNT, test results are needed for two fish including an early life stage, a cold-water species and a species resident in Ontario, and one invertebrate other than a crustacean.

Further data on mutagenicity and taste and odour in water also are needed as specified in OMOE (1992a).

## 8.0 OBJECTIVES OF OTHER AGENCIES

Comparable aquatic environmental objectives (i.e. designed to protect all aquatic species indefinitely) of other agencies were not available. Table 13 lists Quebec's *provisional aquatic criteria* developed using their ministry protocol.

Table 13. *Objectives of other agencies.*

| Dinitrotoluene | Ministère de l'Environnement et de la Faune (Québec) | |
|---|---|---|
| | acute (ug/L) | chronic (ug/L) |
| 2,6-DNT | 900 | 40 |
| 2,4-DNT | 1200 | 20 |
| 3,5-DNT | 1100 | 50 |
| 2,3-DNT | 17 | 0.7 |
| 2,5-DNT | 70 | 3 |
| 3,4-DNT | 80 | 3 |

## 9.0 REFERENCES

References are listed alphabetically. Those followed by a non-sequential number in brackets contain toxicity data listed in Tables 4 and 5. The number in brackets identifies the reference in the tables by the *reference number*.


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Table 2. *Physical-chemical properties of 2,4-DNT.*

| **Compound:** | **Chemical formula:** | **CAS number:** |
|---|---|---|
| 2,4-dinitrotoluene | $C_6H_3CH_3(NO_2)_2$ | 121-14-2 |

| **Properties** (at standard temperature and pressure) | |
|---|---|
| Molecular weight (MW) | 182.14 g/mol[a] |
| Melting point | 64-66°C[a] |
| Boiling point | 300°C[a] |
| Physical state | solid |
| Dissociation constant | not applicable |
| Density (D) | 1.321 g/cm$^3$ [a] |
| Molar volume (MW/D) | 138 cm$^3$/mol |
| Vapour pressure ($P_s$) | 1.5 x 10$^{-2}$ Pa[?] |
| Water solubility ($C_s$) | 1.67 mol/m$^3$ (300 mg/L)[a] |
| Henry's Law Constant ($P_s/C_s$) | 1 x 10$^{-2}$ Pa x m$^3$/mol |

| **Octanol-water partition coefficient (Kow)** | |
|---|---|
| range of literature values of log Kow | 2.0[b] |
| final chosen log Kow value | 2.0 |

| **Baseline uncertainty factor for objective development** |
|---|
| if log Kow < 4, use 1000 |
| if log Kow ≥ 4, use 10 000 |
| baseline uncertainty factor = 1000 |

[a] Dean (1985)
[b] Deneer *et al.* (1987)

Table 3. *Physical-chemical properties of 2,6-DNT.*

| **Compound:** | **Chemical formula:** | **CAS number:** |
|---|---|---|
| 2,6-dinitrotoluene | $C_6H_3CH_3(NO_2)_2$ | 606-20-2 |

| **Properties** (at standard temperature and pressure) | |
|---|---|
| Molecular weight (MW) | 182.14 g/mol[a] |
| Melting point | 64-66°C[a] |
| Boiling point | 285°C[b] |
| Physical state | solid |
| Dissociation constant | not applicable |
| Density (D) | 1.283 g/cm$^3$ [a] |
| Molar volume (MW/D) | 142 cm$^3$/mol |
| Vapour pressure ($P_s$) | 4.6 x 10$^{-2}$ Pa[c] |
| Water solubility ($C_s$) | 1.00 mol/m$^3$ (180 mg/L)[d] |
| Henry's Law Constant ($P_s/C_s$) | 4.6 x 10$^{-2}$ Pa x m$^3$/mol |

| **Octanol-water partition coefficient (Kow)** | |
|---|---|
| range of literature values of log Kow | 2.0[e] |
| final chosen log Kow value | 2.0 |

| **Baseline uncertainty factor for objective development** |
|---|
| if log Kow < 4, use 1000 |
| if log Kow ≥ 4, use 10 000 |
| baseline uncertainty factor = 1000 |

[a] Dean (1985)
[b] CCREM (1987)
[c] USEPA (1986)
[d] Anon (1987)
[e] Deneer *et al.* (1987)

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| **Bluegill sunfish** (*Lepomis macrochirus*) | | | | | | | | |
| juvenile | 1d-LC50 | 27.7 | a/s/? | 17 | ? | ? | ? | 14 |
| " | 1d-LC50 | 33.0 | a/s/? | 21 | ? | ? | 47 | 14 |
| " | 1d-LC50 | 16.4 | a/s/? | 24 | ? | ? | ? | 14 |
| " | 1d-LC50 | 32.5 | a/s/? | ? | ? | ? | 108 | 14 |
| " | 1d-LC50 | 31.5 | a/s/? | ? | ? | ? | 240 | 14 |
| " | 1d-LC50 | 30.2 | a/f/m | 20.8 | 7.2 | 7.9 | 27 | 15 |
| " | 2d-LC50 | 29.9 | a/f/m | 20.8 | 7.2 | 7.9 | 27 | 15 |
| " | 4d-LC50 | 16.0 | a/f/m | 20.8 | 7.2 | 7.9 | 27 | 15 |
| " | 4d-LC50 | 15.7 | a/s/? | ? | ? | ? | ? | 14 |
| " | 4d-LC50 | 23.2 | a/s/? | 17 | ? | ? | ? | 14 |
| " | 4d-LC50 | 24.0 | a/s/? | 17.3 | 7.1 | ? | 45.7 | 15 |
| " | 4d-LC50 | 12.8 | a/s/? | 20.8 | 7.1 | ? | 47 | 14; 15 |
| " | 4d-LC50 | 8.6 | a/s/? | 24 | ? | ? | ? | 14 |
| " | 4d-LC50 | 7.8 | a/s/? | 24.5 | 7.1 | ? | 45.7 | 15 |
| " | 4d-LC50 | 8.4 | a/s/? | 20.3 | 6.3 | ? | 41.7 | 15 |
| " | 4d-LC50 | 9.4 | a/s/? | 20.3 | 8.0 | ? | 41.7 | 15 |
| " | 4d-LC50 | 18.8 | a/s/? | 20.7 | 7.2 | ? | 109 | 14; 15 |
| " | 4d-LC50 | 16.4 | a/s/? | 20.7 | 7.2 | ? | 251 | 14; 15 |
| " | 4d-LC50 | 13.5 | a/s/? | ? | ? | ? | ? | 15 |
| 4.8 cm, 1.21 g | 14d-LC50 | 9.2 | c/f/m | 20.8 | 7.2 | 7.9 | 27 | 15 |
| 8 wks, 2-5 cm/ growth weight | 56d-LOEC | 0.5 | c/f/m | 21 | 7.6 | 8.0 | ? | 14 |
| 8 wks, 2-5 cm/ growth weight | 56d-LOEC[g] | 0.05 | c/f/m | 21 | 7.6 | 7.8 | ? | 14 |

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| **Fathead minnow** (*Pimephales promelas*) | | | | | | | | |
| 90d-juvenile | 1d-LC50 | 36.5 | a/f/? | ? | ? | ? | ? | 14 |
| " | 1d-LC50 | 37.7 | a/f/m | 20.5 | 7.2 | 8.4 | 31 | 15 |
| " | 2d-LC50 | 36.5 | a/f/? | ? | ? | ? | ? | 14 |
| " | 2d-LC50 | 37.7 | a/f/m | 20.5 | 7.2 | 8.4 | 31 | 15 |
| " | 4d-LC50 | 36.5 | a/f/? | ? | ? | ? | ? | 14 |
| " | 4d-LC50 | 36.1 | a/f/m | 20.5 | 7.2 | 8.4 | 31 | 15 |
| " | 4d-LC50 | 34.6 | a/s/? | ? | ? | ? | ? | 14 |
| " | 4d-LC50 | 31.4 | a/s/m | ? | ? | ? | ? | 15 |
| " | 4d-LC50 | 32.5 | a/s/u | 20 | 7.2-8.6 | 26 | 24 | 15 |
| 2.4 cm, 0.28 g | 4d-LC50 | 32.8 | a/s/u | 20 | 7.7 | 7.2 | 34 | 4; 15; 16 |
| 90d-juveniles | 4d-LC50 | 28.5 | a/s/? | ? | ? | ? | ? | 15 |
| 3.2 cm, 0.34 g | 14d-LC50 | 26.0 | c/f/m | 20.5 | 7.2 | 8.4 | 31 | 15 |
| ? | 14d-LC50 | 26.1 | c/f/? | ? | ? | ? | ? | 14 |
| fry/body length | 30d-LOEC | 1.75 | c/?/? | ? | ? | ? | ? | 14 |
| fry/body length | 30d-LOEC | 3.50 | c/?/? | ? | ? | ? | ? | 14 |
| fry/survival | 90d-LOEC | 7.0 | c/?/? | ? | ? | ? | ? | 14 |
| fry/survival | 150d-LOEC | 7.0 | c/?/? | ? | ? | ? | ? | 14 |
| **Channel catfish** (*Ictalurus punctatus*) | | | | | | | | |
| juvenile | 1d-LC50 | 34.7 | a/s/? | 20 | ? | ? | ? | 14 |
| " | 1d-LC50 | 35.8 | a/f/? | ? | ? | ? | ? | 14 |
| " | 1d-LC50 | 36.7 | a/f/m | 20.0 | 7.1 | 7.6 | 25 | 15 |
| " | 2d-LC50 | 35.1 | a/f/? | ? | ? | ? | ? | 14 |
| " | 2d-LC50 | 33.8 | a/f/m | 20.0 | 7.1 | 7.6 | 25 | 15 |
| " | 4d-LC50 | 32.0 | a/f/m | 20.0 | 7.1 | 7.6 | 25 | 15 |

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| " | 4d-LC50 | 29.9 | a/f/? | ? | ? | ? | ? | 14 |
| " | 4d-LC50 | 28.8 | a/s/? | 20 | ? | ? | ? | 14 |
| " | 4d-LC50 | 24.8 | a/s/? | ? | ? | ? | ? | 15 |
| ? | 14d-LC50 | 15.6 | c/f/? | ? | ? | ? | ? | 14 |
| 6.1 cm, 1.98 g | 14d-LC50 | 16.4 | c/f/m | 20.0 | 7.1 | 7.6 | 25 | 15 |
| **Rainbow trout** (*Oncorhynchus mykiss*) | | | | | | | | |
| juvenile | 1d-LC50 | 19.6 | a/f/m | 13.0 | 7.6 | 9.5 | 17 | 15 |
| " | 2d-LC50 | 19.3 | a/f/m | 13.0 | 7.6 | 9.5 | 17 | 15 |
| " | 4d-LC50 | 14.0 | a/f/? | ? | ? | ? | ? | 14 |
| " | 4d-LC50 | 13.9 | a/f/m | 13.0 | 7.6 | 9.5 | 17 | 15 |
| " | 4d-LC50 | 13.8 | a/s/? | ? | ? | ? | ? | 15 |
| 6.3 cm, 2.77 g | 14d-LC50 | 6.3 | c/f/m | 13.0 | 7.6 | 9.5 | 17 | 15 |
| ? | 14d-LC50 | 6.8 | c/f/? | ? | ? | ? | ? | 14 |
| **Guppy** (*Poecelia reticulata*) | | | | | | | | |
| 4 week old | 4d-LC50 | 25.0 | a/?/? | ? | ? | ? | ? | 1 |
| 2-3 months old | 14d-LC50 | 12.6 | c/r/m | 21-22 | 6.8-7.2 | >4.5 | 25 | 11 |
| **Cladoceran** (*Daphnia magna*) | | | | | | | | |
| < 12h old | 1d-LC50 | 31.2 | a/f/m | 20.0 | 7.5 | 8.3 | 20 | 15 |
| ? | 1d-LC50 | 22.0 | a/s/? | ? | ? | ? | ? | 5 |
| immobilization | 1d-EC50 | 22.0 | a/s/? | ? | ? | ? | ? | 9 |
| < 12h old | 2d-LC50 | 30.4 | a/f/m | 20.0 | 7.5 | 8.3 | 20 | 15 |
| < 12h old | 2d-LC50 | 38.3 | a/s/? | ? | ? | ? | ? | 3; 14; 15 |
| < 12h old | 2d-LC50 | 47.5 | a/s/u | 20 | 7.7 | ? | 34 | 15; 16 |
| immobilization | 2d-EC50 | 35.0 | a/s/u | 20 | 7.9 | ? | 26 | 24 |

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| < 24h old immobilization | 2d-EC50 | 34.0 | a/s/u | 20 | 8.4 | ? | 200 | 12 |
| mobility | 2d-EC50 | 10-16 | a/s/? | ? | ? | ? | ? | 1 |
| < 12h old | 4d-LC50 | 23.9 | a/f/m | 20.0 | 7.5 | 8.3 | 20 | 15 |
| < 12h old | 14d-LC50 | 4.1 | c/f/m | 20.0 | 7.5 | 8.3 | 20 | 3; 15 |
| < 24h old immobilization | 21d-EC50 | 0.6 | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| < 24h old pop. growth | 21d-LOEC | 1.0 | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| < 24h old body length | 21d-LOEC | 1.0 | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| **Oligochaete worm** (*Lumbriculus variegatus*) | | | | | | | | |
| ? | 1d-LC50 | 99.5 | a/f/m | 21.0 | 7.3 | 8.2 | 45 | 14; 15 |
| " | 2d-LC50 | 80.9 | a/f/m | 21.0 | 7.3 | 8.2 | 45 | 15 |
| " | 4d-LC50 | 47.2 | a/f/m | 21.0 | 7.3 | 8.2 | 45 | 15 |
| " | 4d-LC50 | 50.7 | a/f/? | ? | ? | ? | ? | 14 |
| " | 14d-LC50 | 31.1 | c/f/? | ? | ? | ? | ? | 14 |
| " | 14d-LC50 | 30.4 | c/f/m | 21.0 | 7.3 | 8.2 | 45 | 15 |
| " | 14d-LC50 | 32.4 | c/f/? | ? | ? | ? | ? | 3 |
| **Midge** (*Tanytarsus dissimilis*) | | | | | | | | |
| ? | 2d-LC50 | 22.5 | a/s/? | ? | ? | ? | ? | 14; 15 |
| **Duckweed** (*Lemna perpusilla*) | | | | | | | | |
| colony growth | 11d-LOEC | 0.1 | c/?/? | ? | 6.3 | ? | ? | 25 |
| " | 11d-LOEC | 1.0 | c/?/? | ? | 8.5 | ? | ? | 25 |
| **Green alga** (*Selenastrum capricornutum*) | | | | | | | | |
| cell multiplication | 4d-EC50 | 1.1 | c/?/? | ? | ? | ? | ? | 14 |
| " | 14d-EC50 | 3.3 | c/?/? | ? | ? | ? | ? | 3; 14 |

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| " | 4d-LOEC | 0.9 | c/s/? | ? | ? | ? | ? | 15 |
| " | 14d-LOEC | 4.7 | c/s/? | ? | ? | ? | ? | 15 |
| **Diatom** (*Navicula pelliculosa*) | | | | | | | | |
| " | 14d-EC50 | 8.0 | c/?/? | ? | ? | ? | ? | 14 |
| " | 14d-LOEC | 9.8 | c/s/? | ? | ? | ? | ? | 15 |
| **Bluegreen alga** (*Microcystis aeruginosa*) | | | | | | | | |
| " | 4d-LOEC | 0.5 | c/s/? | ? | ? | ? | ? | 15 |
| " | 14d-LOEC | 0.5 | c/s/? | ? | ? | ? | ? | 15 |
| " | 8d-threshold | 0.13 | c/s/? | ? | ? | ? | ? | 6; 27 |
| **Bluegreen alga** (*Anaebena flos-aquae*) | | | | | | | | |
| " | 14d-EC50 | 10.2 | c/?/? | ? | ? | ? | ? | 14 |
| " | 14d-LOEC | 0.9 | c/s/? | ? | ? | ? | ? | 15 |
| **Green alga** (*Chlorella pyrenoidosa*) | | | | | | | | |
| " | 4d-EC50 | 0.9 | c/s/u | ? | ? | ? | ? | 12 |
| **Green alga** (*Scenedesmus quadricauda*) | | | | | | | | |
| " | 8d-threshold | 2.7 | c/?/? | 27 | ? | ? | ? | 6; 7; 27 |
| **Green alga** (*Scenedesmus pannonicus*) | | | | | | | | |
| " | 4d-EC50 | 2.3 | c/?/? | ? | ? | ? | ? | 1 |
| **Protozoa** (*Chilomonas paramecium*) | | | | | | | | |
| " | 3d-threshold | 0.13 | c/s/? | ? | ? | ? | ? | 8 |
| **Protozoa** (*Entosiphon sulcatum*) | | | | | | | | |
| " | 3d-threshold | 0.98 | c/s/? | 25 | ? | ? | ? | 7; 8; 27 |
| **Protozoa** (*Uronema parduczi*) | | | | | | | | |
| " | 3d-threshold | 0.55 | c/s/? | ? | ? | ? | ? | 8; 27 |
| **Bacteria** (*Pseudomonas putida*) | | | | | | | | |

Table 4. *Aquatic Toxicity data for 2,4-DNT. (footnotes at end of Table 5)*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | DO | H | |
| " | 16h-threshold | 57.0 | c/s/? | 25 | ? | ? | ? | 7; 27 |

Table 5. *Aquatic Toxicity data for 2,6-DNT.*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | D.O. | H | |
| **Fathead minnow** (*Pimephales promelas*) | | | | | | | | |
| juveniles: 2.4 cm, 0.28 g | 4d-LC50 | 18.5 | a/s/u | 20 | 7.7 | 7.2 | 34 | 4; 15; 16 |
| 90d-juveniles | 4d-LC50 | 19.8 | a/s/? | 20 | 7.2-8.6 | ? | 26 | 2; 24 |
| **Guppy** (*Poecelia reticulata*) | | | | | | | | |
| 2-3 months old | 14d-LC50 | 17.8 | c/r/m | 21-22 | 6.8-7.2 | >4.5 | 25 | 11 |
| **Cladoceran** (*Daphnia magna*) | | | | | | | | |
| ? | 1d-LC50 | 14.0 | a/s/? | ? | ? | ? | ? | 5 |
| immobilization | 1d-EC50 | 21.0 | a/s/? | ? | ? | ? | ? | 9 |
| <24h old immobilization | 2d-EC50 | 21.8 | a/s/u | 20 | 7.7 | ? | 34 | 15; 16 |
| " | 2d-EC50 | 21.7 | a/s/u | 20 | - | ? | 26 | 2; 24 |
| " | 2d-EC50 | 34.0 | a/s/u | 20 | 8.4 | ? | 200 | 12 |
| " | 21d-EC50 | 9.6 | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| < 24h old pop. growth | 21d-LOEC | 10.0 | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| < 24h old body length | 21d-LOEC | 1.0[g] | c/r/u | 20 | 8.4 | ? | 200 | 12 |
| **Bluegreen alga** (*Microcystis aeruginosa*) | | | | | | | | |

Table 5. *Aquatic Toxicity data for 2,6-DNT.*

| Species stage and response[a] | Toxicity test end-point[b] | Water conc.[c] (mg/L) | Test type[d] | Test conditions[e] | | | | Ref. no.[f] |
|---|---|---|---|---|---|---|---|---|
| | | | | T | pH | D.O. | H | |
| cell multiplication | 8d-threshold | 0.5 | c/s/u | ? | ? | ? | ? | 6; 27 |
| **Green alga** (*Chlorella pyrenoidosa*) | | | | | | | | |
| " | 4d-EC50 | 6.8 | c/s/u | ? | ? | ? | ? | 12 |
| **Green alga** (*Scenedesmus quadricauda*) | | | | | | | | |
| " | 8d-threshold | 12 | c/s/? | 27 | ? | ? | ? | 6; 7; 27 |
| **Protozoa** (*Entosiphon sulcatum*) | | | | | | | | |
| " | 3d-threshold | 11 | c/s/? | 25 | ? | ? | ? | 2; 7; 27 |
| **Protozoa** (*Uronema parduczi*) | | | | | | | | |
| " | 3d-threshold | 23.0 | c/s/? | ? | ? | ? | ? | 2; 8; 27 |
| **Protozoa** (*Tetrahymena pyriformis*) | | | | | | | | |
| | 1d-EC50 | 100 | c/s/? | 30 | ? | ? | ? | 29 |
| **Bacteria** (*Pseudomonas putida*) | | | | | | | | |
| " | 16h-threshold | 26 | c/s/? | 25 | ? | ? | ? | 2,7; 27 |

Footnotes:

[a] Lethality unless indicated otherwise. The thresholds were inhibition of cell multiplication. [b] LC50 = median lethal concentration; EC50 (IC50) = effective (inhibitory) concentration; LOEC = lowest observed effect concentration; thresholds are similar to no observed effect concentrations (NOECs). [c] Water concentration. [d] a = acute; c = chronic/s = static; r = replacement; f = flowthrough; m = measured water concentrations; u= unmeasured concentrations. ? = unknown. [e] T = temperature in degrees C; D.O. = dissolved oxygen concentration in mg/L; H = hardness in mg/L as $CaCO_3$. [f] A reference number identifies a cited reference with the corresponding number in brackets at the end of the citation. [g] The lowest effect concentration - critical value for objective setting.

Table 9. *Bioconcentration factors for 2,4-DNT.*

| Species | Tissue | Exposure period | | |
|---|---|---|---|---|
| | | 2d | 4d | 14d |
| **Guppy** (*P. reticulata*) | | | | |
| | whole body[1] | | 16 (22) | |
| **Bluegill sunfish** (*L. macrochirus*) | | | | |
| | whole body[2] | | | 25 |
| | brain[2] | | | 103 |
| | kidney[2] | | | 48 |
| | G.I. tract[2] | | | 29 |
| | liver[2] | | | 29 |
| | gills[2] | | | 24 |
| | muscle[2] | | | 11 |
| | viscera[3] | 53, 47 | 78, 84 | |
| | muscle[3] | 4, 5 | 4, 5 | |
| **Cladoceran** (*D. magna*) | | | | |
| | whole body[3] | | 13, 14 | |
| **Oligochaete worm** (*L. variegatus*) | | | | |
| | whole body[3] | | 58, 63 | |
| **Green alga** (*S. capricornutum*) | | | | |
| | whole cell[3] | | 2507, 2149 | |

1 Deneer *et al.* (1987). Water concentration 2 to 4 mg/L. Female fish 5 to 8 months old, wet weight 60 to 450 mg, fat content 8 %. BCF value (22) is for 2,6-DNT.

2 Hartley (1981). Water concentration = 3 mg/L.

3 Liu *et al.* (1983a; 1983b). Water concentration = 1.0 to 1.3 mg/L of $14^{C}$-radiolabelled chemical. BCF value after comma is 2,4-DNT in a synthetic wastewater mixture.

## Table 10: UNCERTAINTY FACTOR WORKSHEET

| CHEMICAL: | CAS No.: | CONCENTRATION UNITS: |
|---|---|---|
| **2,4-DINITROTOLUENE** | 141-14-2 | mg/L |

| Test Conditions | | Species | Toxicity End-point | Effect Conc. | Data Codes | Data Type | Calibration Factor | Reference |
|---|---|---|---|---|---|---|---|---|
| ACUTE | VERTEBRATE | Bluegill sunfish | lethality | 16.0 | F,M | 1° | 0.8 | Liu *et al.* 1983a |
| | | Fathead minnow | lethality | 36.1 | F, M | 1° | 0.8 | Liu *et al.* 1983a |
| | | Rainbow trout | lethality | 13.9 | F, M | 1° | 0.8 | Liu *et al.* 1983a |
| | INVERT | *Daphnia magna* | lethality | 23.9 | F, M | 1° | 0.8 | Liu *et al.* 1983a |
| | | Oligochaete worm | lethality | 47.2 | F, M | 1° | 0.8 | Liu *et al.* 1983a |
| CHRONIC | VERTEBRATE | Bluegill sunfish | growth | 0.05 | F, M | 1° | 0.5 | Liu *et al.* 1983a |
| | | Fathead minnow | lethality | 26.0 | F, M | 1° | 0.5 | Liu *et al.* 1983a |
| | | Rainbow trout | lethality | 6.3 | F, M | 1° | 0.5 | Liu *et al.* 1983a |
| | INVERT | *Daphnia magna* | lethality | 4.1 | F, M | 1° | 0.5 | Liu *et al.* 1983a |
| | | Oligochaete worm | lethality | 30.4 | F, M | 1° | 0.5 | Liu *et al.* 1983a |
| | PLANT | Bluegreen algae (*Anaebena flos-aquae*) | population growth | 0.9 | S, ? | 2° | 0.9 | Liu *et al.* 1983a |

CALCULATION OF FINAL UNCERTAINTY FACTOR:

Since Log Kow < 4.0, the baseline uncertainty factor = 1000

Baseline Uncertainty Factor x Calibration Factors (maximum number = 11)

1000 x 0.8 x 0.8 x 0.8 x 0.8 x 0.8 x 0.5 x 0.5 x 0.5 x 0.5 x 0.9

13 Final uncertainty factor (not to be less than a value of 13)

Critical value ÷ Final uncertainty factor = Interim PWQO

= 0.05 ÷ 13 = 0.004 mg/L

S = static; R = renewal; F = flow-through; U = unmeasured conc; M = measured conc.; 1° = Primary; 2° = Secondary; ? = Unknown

## Table 11: UNCERTAINTY FACTOR WORKSHEET

| CHEMICAL: | CAS No.: | CONCENTRATION UNITS: |
|---|---|---|
| **2,6-DINITROTOLUENE** | 606-20-2 | mg/L |

| Test Conditions | | Species | Toxicity End-point | Effect Conc. | Data Codes | Data Type | Calibration Factor | Reference |
|---|---|---|---|---|---|---|---|---|
| ACUTE | VERTEBRATE | Fathead minnow | lethality | 18.5 | S, U | 2° | 0.9 | Liu *et al.* 1983a |
| | | | | | | | | |
| | | | | | | | | |
| | INVERT | Cladoceran (*Daphnia magna*) | lethality | 21.8 | S, U | 2° | 0.9 | Liu *et al.* 1983a |
| | | | | | | | | |
| CHRONIC | VERTEBRATE | Guppy | lethality | 17.8 | R, M | 1° | 0.5 | Deneer *et al.* 1987 |
| | | | | | | | | |
| | | | | | | | | |
| | INVERT | Cladoceran (*Daphnia magna*) | growth | 1.0 | R, U | 2° | 0.7 | Deneer *et al.* 1989 |
| | | Protozoa (*Tetrahymena pyriformis*) | population growth | 100 | S, ? | 2° | 0.7 | Yoshitada, 1985 |
| | PLANT | Bluegreen algae (*Microcystis aeruginosa*) | population growth | 0.5 | S, U | 2° | 0.9 | Bringmann and Kühn, 1978 |

CALCULATION OF FINAL UNCERTAINTY FACTOR:

Since Log Kow < 4.0, the baseline uncertainty factor = 1000

Baseline Uncertainty Factor x Calibration Factors (maximum number = 11)

1000 x 0.9 x 0.9 x 0.5 x 0.7 x 0.7 x 0.9 x [ ] x [ ] x [ ] x [ ] x [ ]

179 Final uncertainty factor (not to be less than a value of 13)

Critical value ÷ Final uncertainty factor = Interim PWQO

= 1.0 ÷ 179 = 0.006 mg/L

S = static; R = renewal; F = flow-through; U = unmeasured conc.; M = measured conc.; 1° = Primary; 2° = Secondary; ? = Unknown

Figure 1. 2,4-DNT Water Quality Objective Derivation Graph

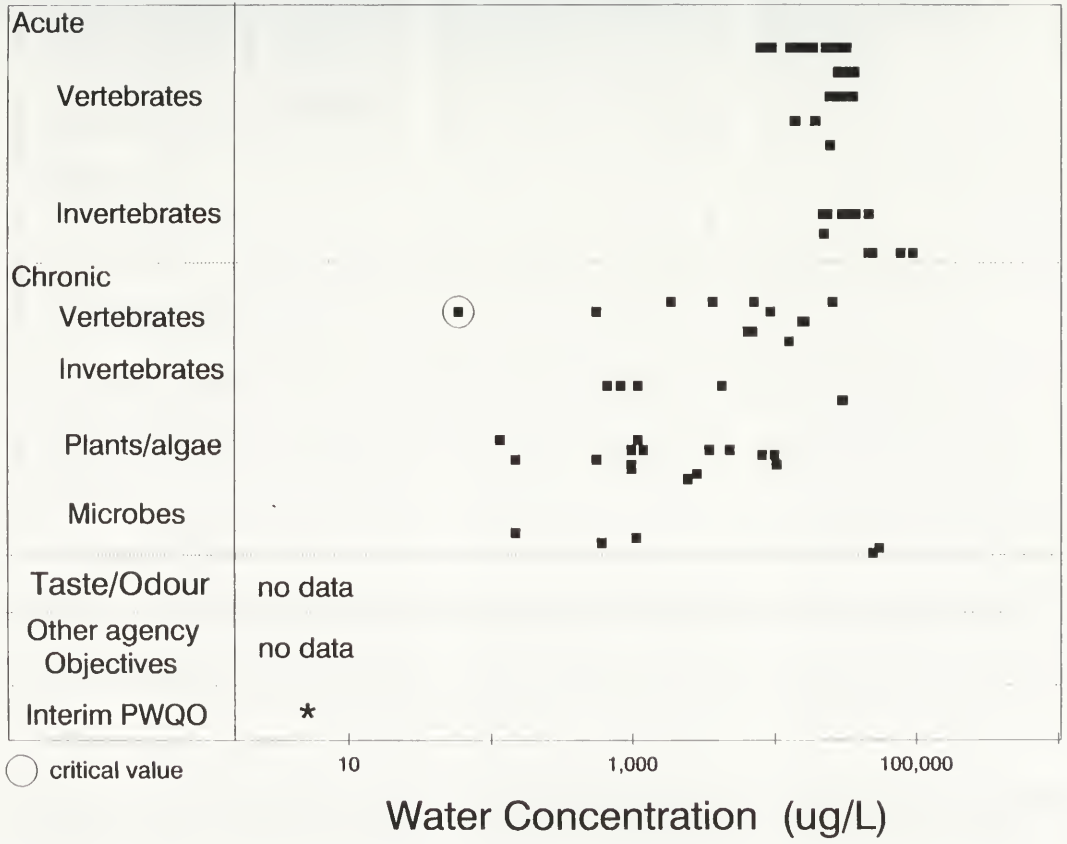
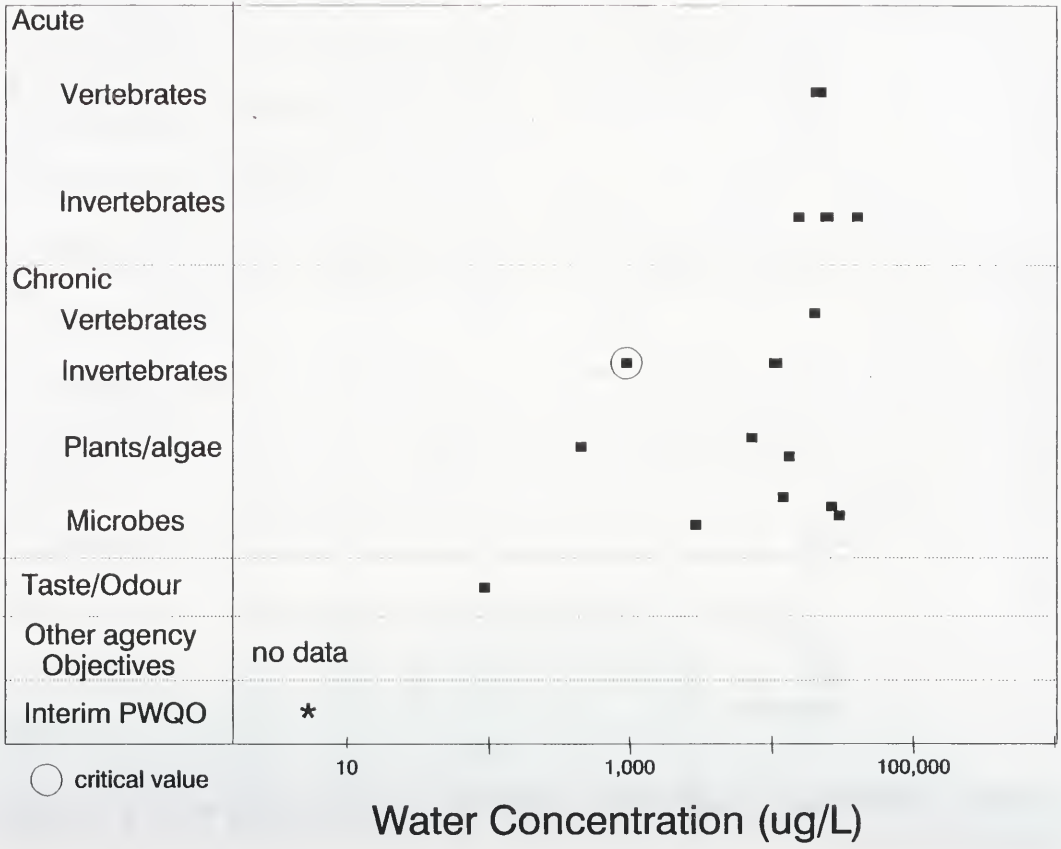


Figure 2. 2,6-DNT Water Quality Objective Derivation Graph



## APPENDIX A:
## MUTAGENICITY

Nitro-aromatic compounds, including 2,4-DNT and 2,6-DNT, tend to be mutagenic with dinitro compounds being more mutagenic than mononitro compounds. Nitrotoluenes are less mutagenic than the nitropyrenes, nitrofluorenes, and nitronaphthalenes. Among the nitrotoluenes, it is the DNT isomers that elicit the predominant mutagenicity and these are generally not strongly mutagenic.

Like other nitro compounds, 2,4-DNT and 2,6-DNT are direct acting compounds (they do not require the standard mammalian microsomal enzymes (S9) to form a reactive product). Indeed, the presence of S9 preparations may reduce the mutagenic potential of these compounds (Couch *et al.*, 1981; Spanggord *et al.*, 1982). Nitro-aromatic compounds, however, do require some activation. In general they are activated to mutagens and other reactive molecules by reduction to arylhydroxylamine intermediates. This reduction is brought about by bacterial nitroreductase enzymes (Araki, *et al.*, 1970; Blumer *et al.*, 1980). These intermediates are further metabolized to their ultimate reactive compounds (toxic, mutagenic and/or carcinogenic endpoints) in bacteria or mammalian cell systems by O-acetyltransferase (Oda *et al.*, 1993).

The normal levels of these enzymes in bacteria are such that most DNT isomers will probably elicit negative or weak mutagenic responses in bacterial assays. When tested on the *Salmonella typhimurium* mammalian microsomal assay (Ames test) with or without mammalian activation enzymes, 2,4-DNT and 2,6-DNT were both weakly positive. 2,5-DNT and 3,5-DNT generally produced a strong positive response while the 3,4-DNT isomer was mostly negative (Simmon *et al.*, 1977; Couch *et al.*, 1981; Tokiwa *et al.*, 1981; Mori *et al.*, 1982; Spanggord *et al.*, 1982). In a comparison of the mutagenic potential of the DNT isomers, Spanggord *et al.* (1982) indicated that 2,4-DNT and 2,6-DNT were four to five times less potent than the 2,5-DNT and 3,5-DNT. None of the isomers produced a positive response with a nitroreductase deficient bacterial strain emphasizing the importance of nitroreductase enzymes.

The mutagenic response of DNT in bacterial assays can be amplified by generating bacterial strains that overproduce nitroreductase enzymes or O-acetyltransferase or by the addition of natural substances such as flavinmononucleotide that help mediate the reduction process. When tested on strains in which the production of nitroreductase and/or O-acetyltransferase has been enhanced, 2,4-DNT and 2,6-DNT produce mutagenic responses 5 to 1200 times greater than with standard *Salmonella* strains TA98 and TA100 (Sayama *et al.*, 1991; Oda *et al.*, 1992, 1993; Einisto *et al.*, 1991; Hagiwara *et al.*, 1993). Somewhat similar increases in the mutagenic responses of DNT were observed after the addition of flavinmononucleotide to a *Salmonella* preincubation assay mixture (Dellarco and Prival, 1989). This amplification does not necessarily distort the mutagenic potential of the nitro compounds.

In standard assays, the bacteria may have only a limited range of nitroreductases while the diversity of bacteria in the gut, where most of the nitroreductase activity occurs in animal models, allows for a potential wide range of nitroreductase activity (Dellarco and Prival, 1989). Because the nitroreduction that would occur naturally within an animal is difficult to predict, the mutagenic potential of nitro compounds such as 2,4-DNT and 2,6-DNT is difficult to quantify.

The mutagenic response of DNT on eucaryotic systems can also be, at least, partially explained by the availability of nitroreductase enzymes. When tested for DNA damage in a rat hepatocyte cell culture *in vitro* assay, all isomers of DNT produced negative results (Bermudez *et al.*, 1979). These results were substantiated using a Chinese hamster ovary mammalian mutation (HGPRT) assay (Abernethy and Couch, 1982). However, when DNT isomers were fed to rats, 2,4-DNT, 2,6-DNT and technical-grade DNT (tDNT) treated animals all elicited significant hepatocellular DNA damage (Mirsalis and Butterworth, 1982).

The primary explanation for this may be the lack of nitroreductases in mammalian tissues, *in vitro*; however, when DNT was fed to the animals, the compounds were available for metabolism by gut bacteria. The importance of gut bacteria in the metabolism of DNT to reactive products was demonstrated by Mirsalis *et al.* (1982). Mirsalis *et al.* (1982) fed tDNT to both normal and axenic (germ free) rats; only in the normal rats was DNA damage detected. In one study with rat hepatocytes in culture both 2,4-DNT and 2,6-DNT did induce DNA damage but only at the highest dose tested (Sina *et al.*, 1983). Dominant lethal studies with 2,4-DNT show limited positive responses at high dose in rats (Hodgson *et al.*, 1976; Borzelleca and Carchman, 1982). All mice data were negative (Soares and Lock, 1980).

A comparison of 2,4-DNT and 2,6-DNT showed that both are more toxic in rats than in mice. This suggests a potential large difference in species metabolic capabilities and, consequently different sensitivities to these compounds among species. This is also demonstrated by the fact that both compounds are rat carcinogens, but not carcinogens to mice; a pattern similar to that seen for the dominant lethal data presented above. 2,6-DNT is a complete hepatocellular carcinogen, having both initiating and promoting capabilities. 2,4-DNT is overall a less potent substance that does not possess initiating capabilities (Popp and Leonard, 1982), and it produces only benign tumours of the skin and mammary gland (NCI, 1978; Dunkel *et al.*, 1985). Yet both isomers bind to liver DNA, with the 2,6-DNT isomer binding much more strongly. The rat hepatocellular carcinomas induced by tDNT were due to the 2,6-DNT component of the mixture, rather than the 2,4-DNT (Popp and Leonard, 1982).

In summary, both 2,4-DNT and 2,6-DNT are potentially hazardous chemicals. Both are weak to moderate mutagens in various assay systems. This mutagenic effect is modulated by the quantity and diversity of nitroreductase and O-acetyltransferase enzymes present. Since there appears to be a significant diversity in the availability of these enzymes in animal systems, the genotoxic effects of DNT would be predicted to differ greatly among animal systems, as shown by the difference in genotoxic responses between rats and mice. This diversity in response makes it difficult to accurately quantify the level of hazard. The data do suggest that these chemicals can pose a hazard with 2,6-DNT more of a threat than 2,4-DNT.

## APPENDIX B:
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